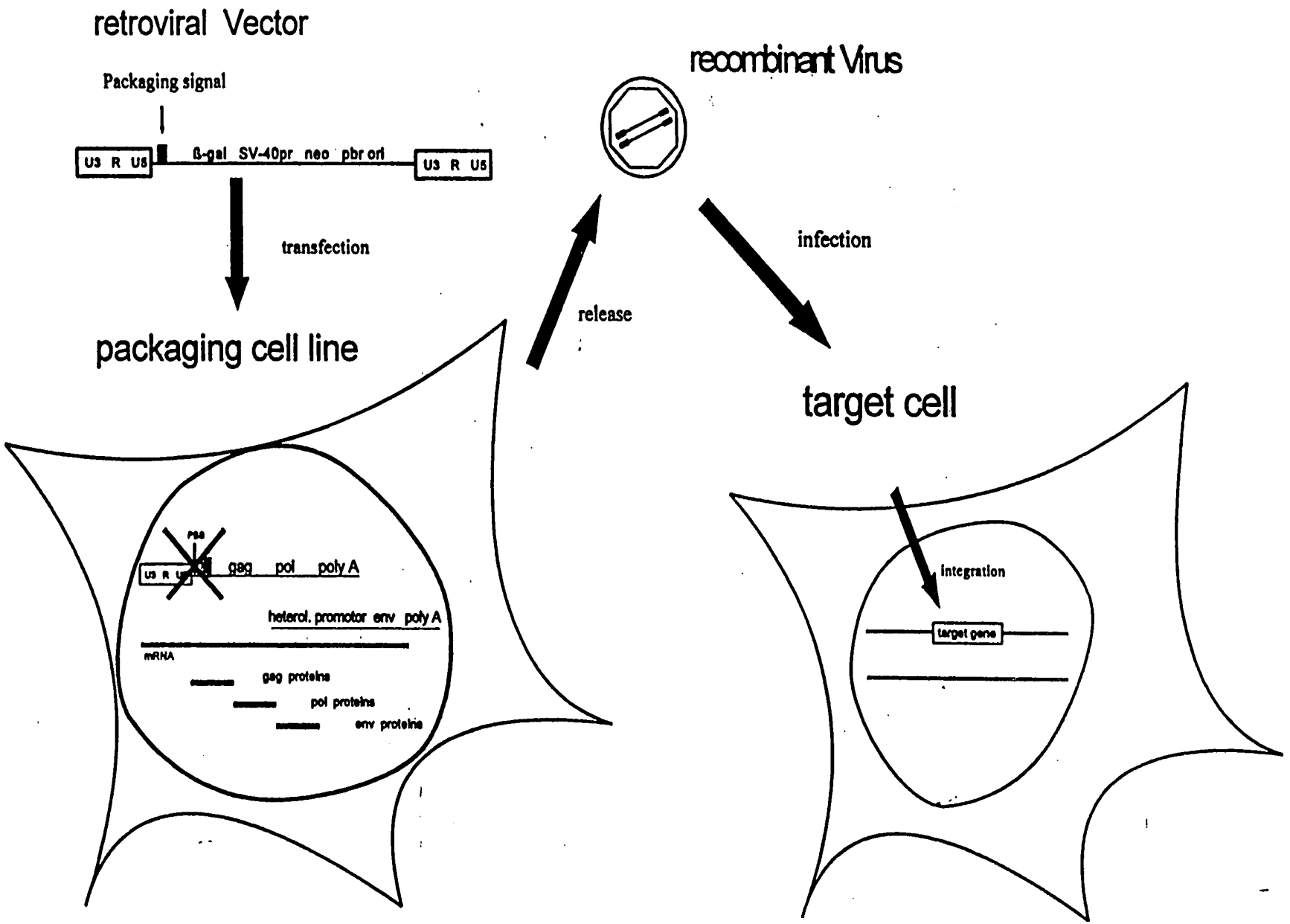
retroviral Vector
Packaging signal
U3 R U5
ß-gal SV-40pr neo pbr ori
U3 R U5
transfection
packaging cell line
recombinant Virus
release
infection
target cell
psi
U3 R U5
gag pol poly A
heterol. promotor env poly A
mRNA
gag proteins
pol proteins
env proteins
integration
target gene

Fig. 1

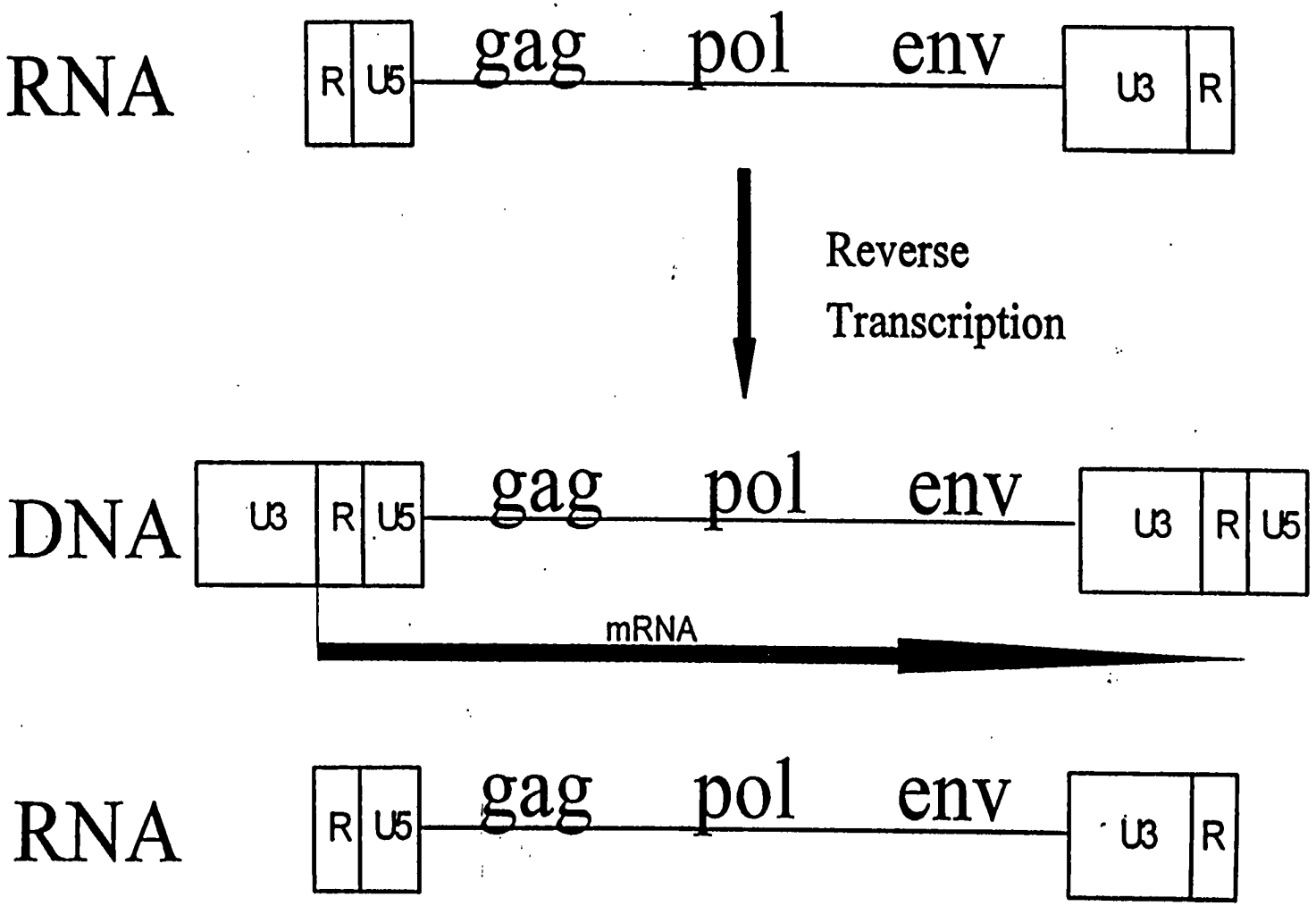
RNA
R
U5
gag
pol
env
U3
R
Reverse
Transcription
DNA
U3
R
U5
gag
pol
env
U3
R
U5
mRNA
RNA
R
U5
gag
pol
env
U3
R

Fig. 2

Construction of a U3 minus BAG-vector (MLV)

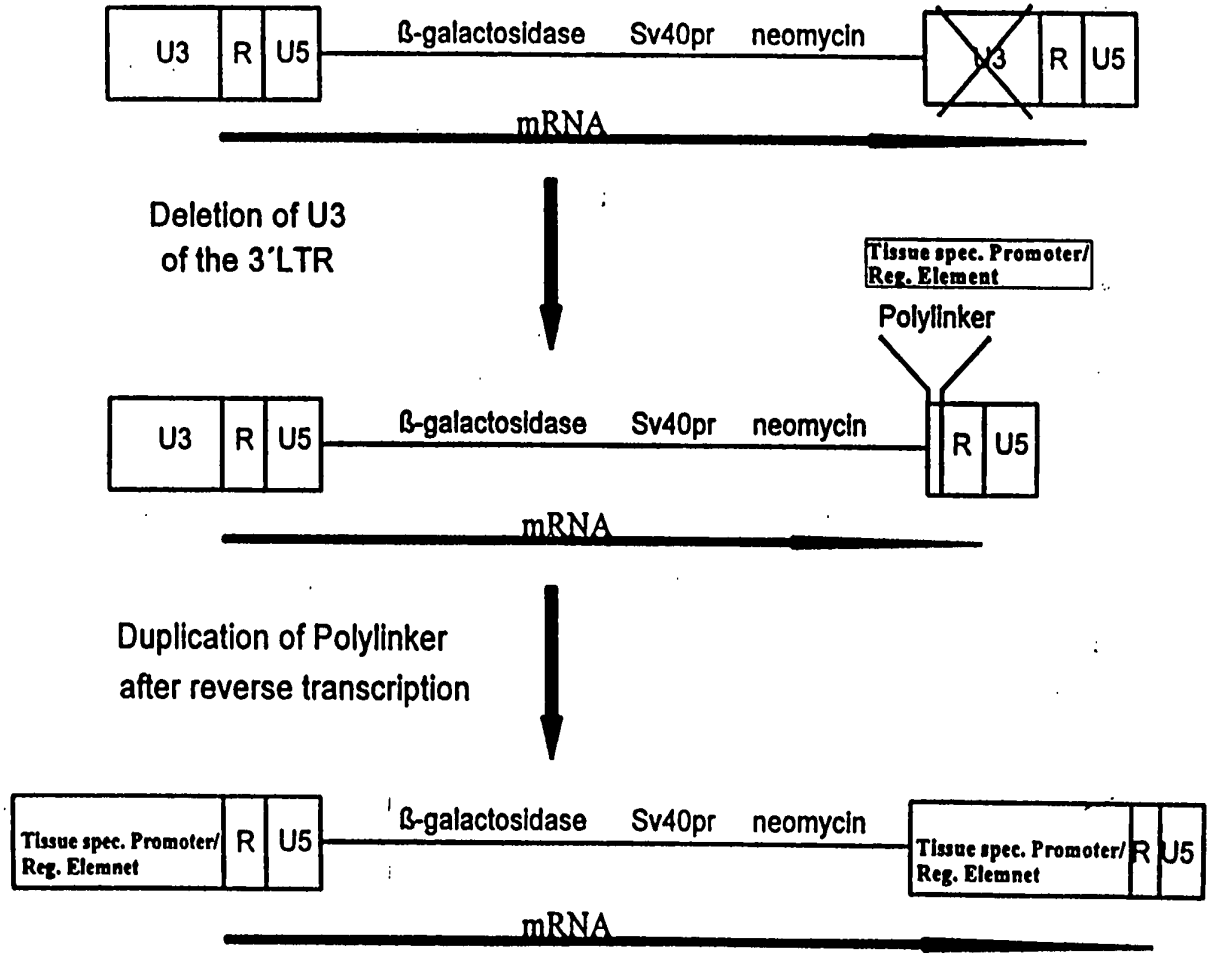

Fig. 3

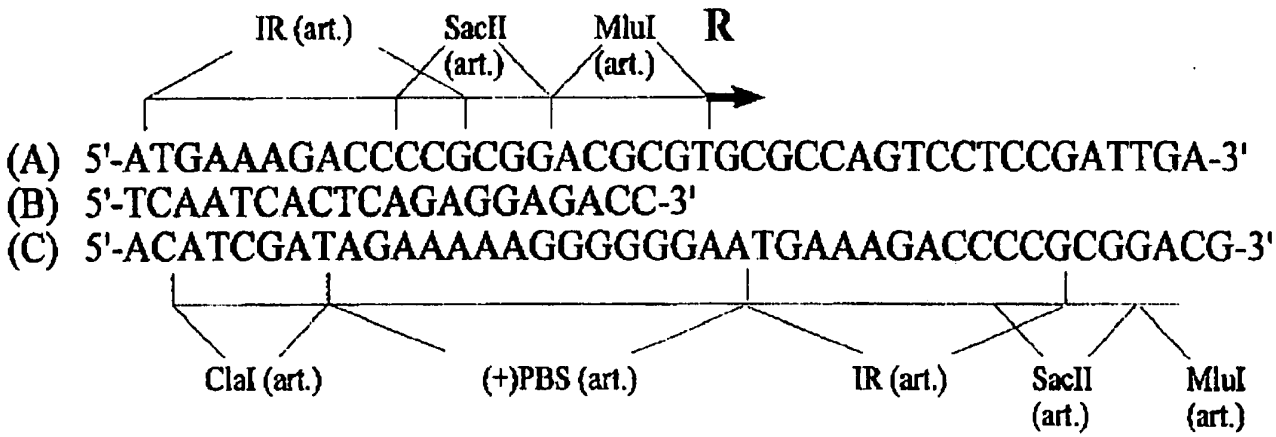
IR (art.)
SacII (art.)
MluI (art.)
R
(A) 5'-ATGAAAGACCCCGCGGACGCGTGCGCCAGTCCTCCGATTGA-3'
(B) 5'-TCAATCACTCAGAGGAGACC-3'
(C) 5'-ACATCGATAGAAAAAGGGGGGAATGAAAGACCCCGCGGACG-3'
ClaI (art.)
(+)PBS (art.)
IR (art.)
SacII (art.)
MluI (art.)

Fig. 4

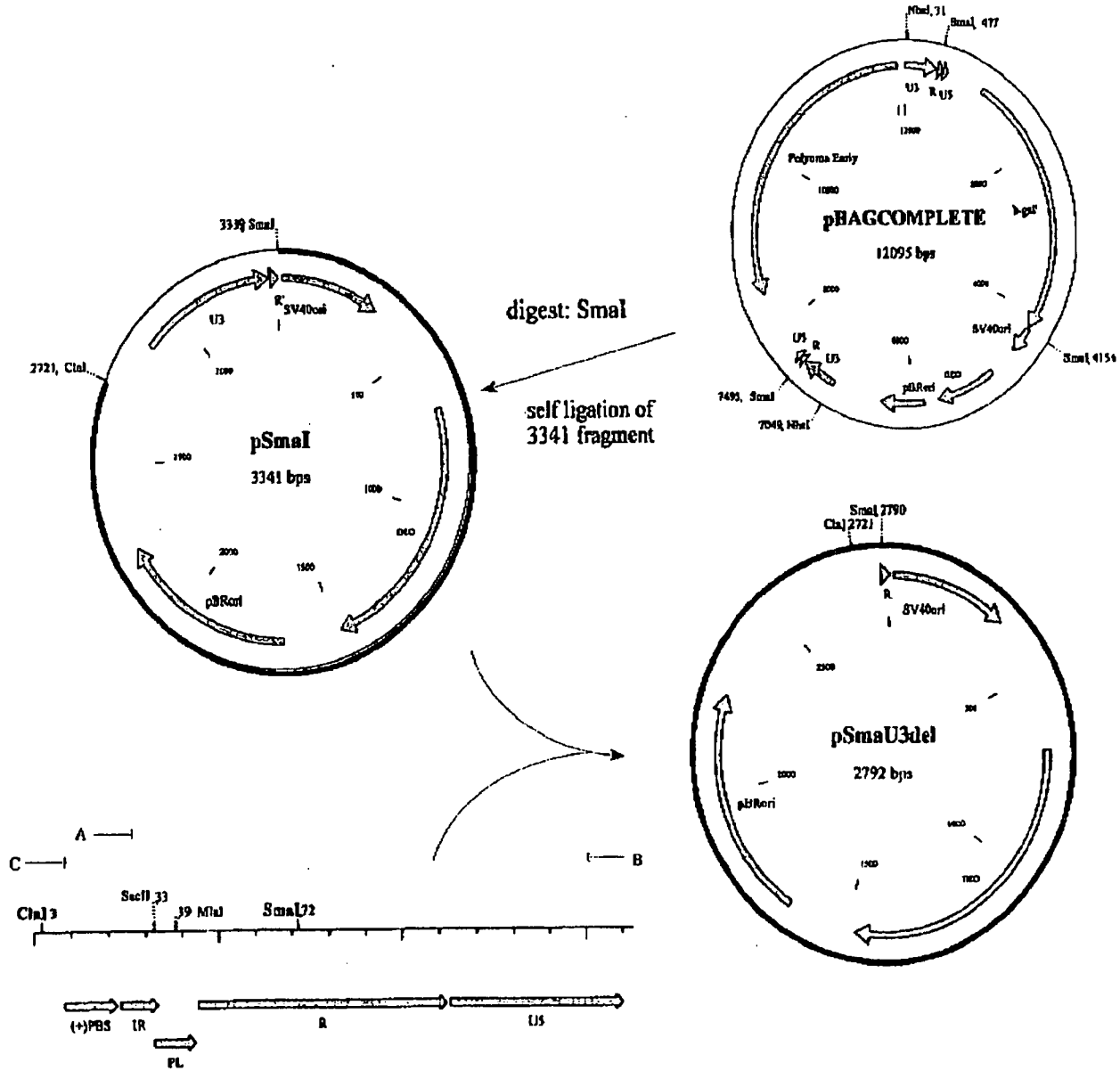
pBAGCOMPLETE
12095 bps
digest: SmaI
self ligation of
3341 fragment
pSmaI
3341 bps
pSmaU3del
2792 bps
A
B
C
ClaI 3
SacII 33
39 MluI
SmaI 72
(+)PBS
IR
R
U5
PL

Fig. 5

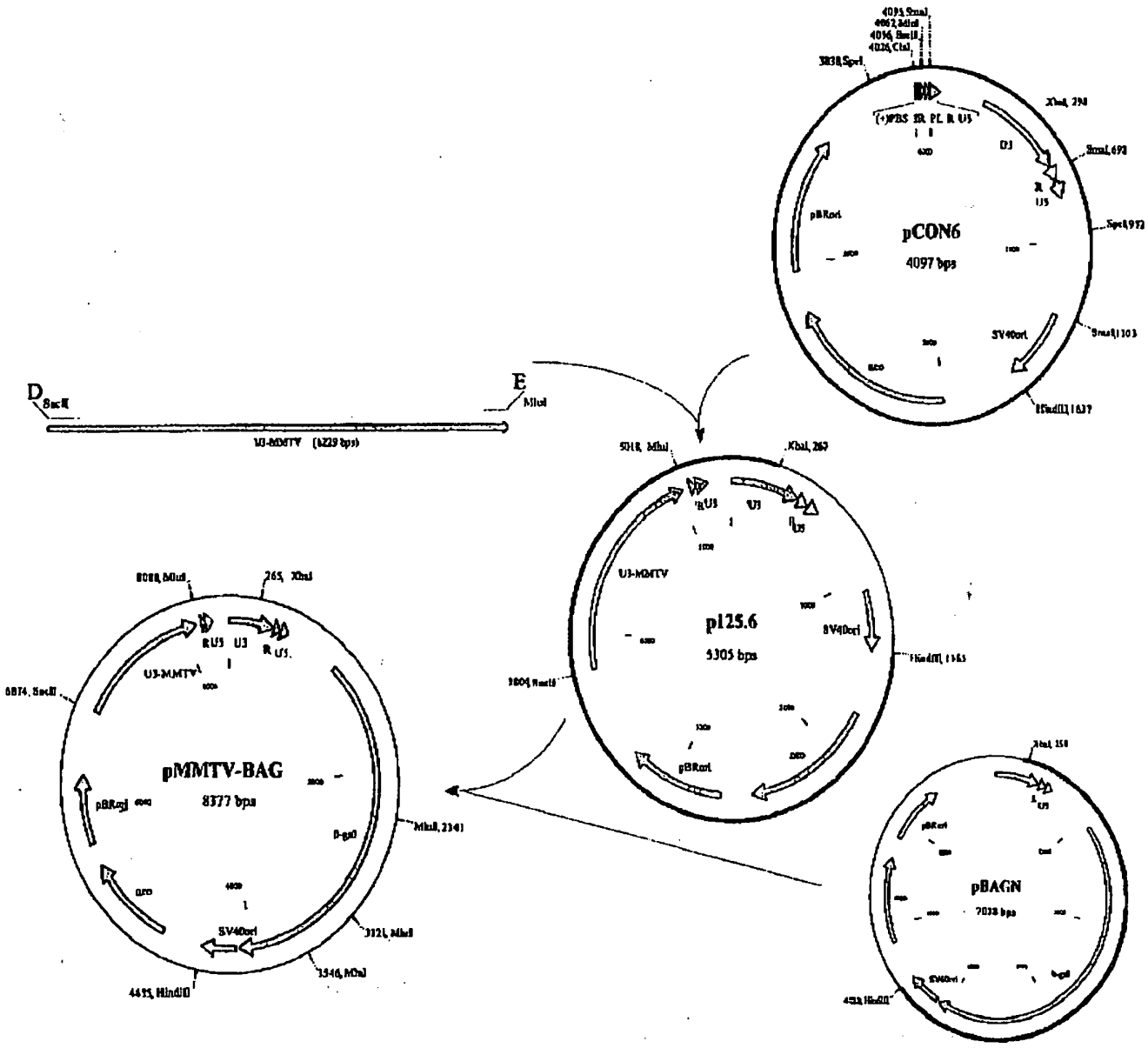
pCON6
4097 bps
pBRori
SV40ori
pBR322ori
pMMTV-BAG
8377 bps
pBRori
SV40ori
pBR322ori
p125.6
5305 bps
U3-MMTV
SV40ori
pBR322ori
pBAGN
7033 bps
SV40ori
pBR322ori

Fig. 8

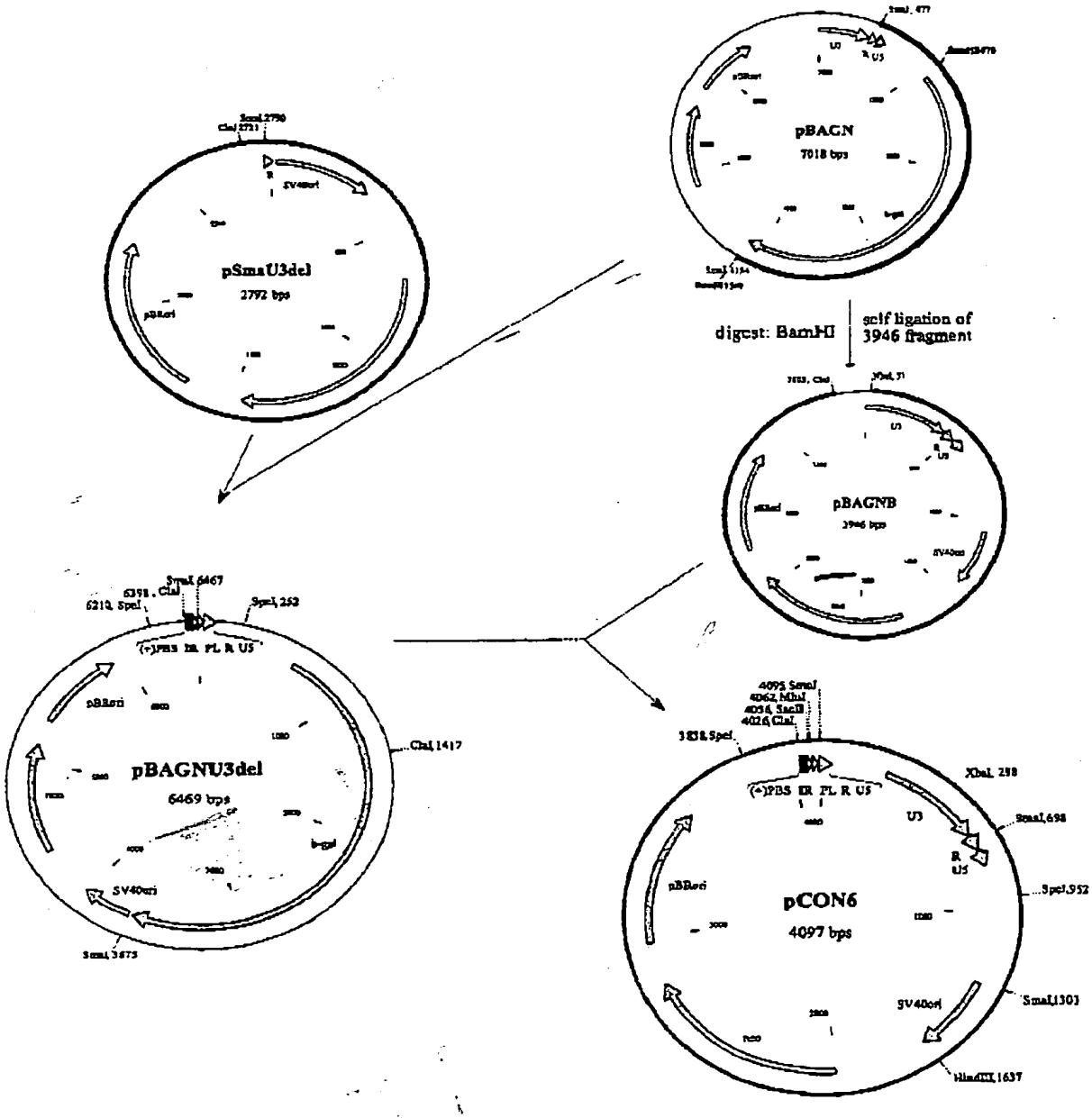
pSmaU3del
2792 bps
pBAGN
7018 bps
digest: BamHI
self ligation of
3946 fragment
pBAGNB
3946 bps
pBAGNU3del
6469 bps
pCON6
4097 bps

Fig. 6

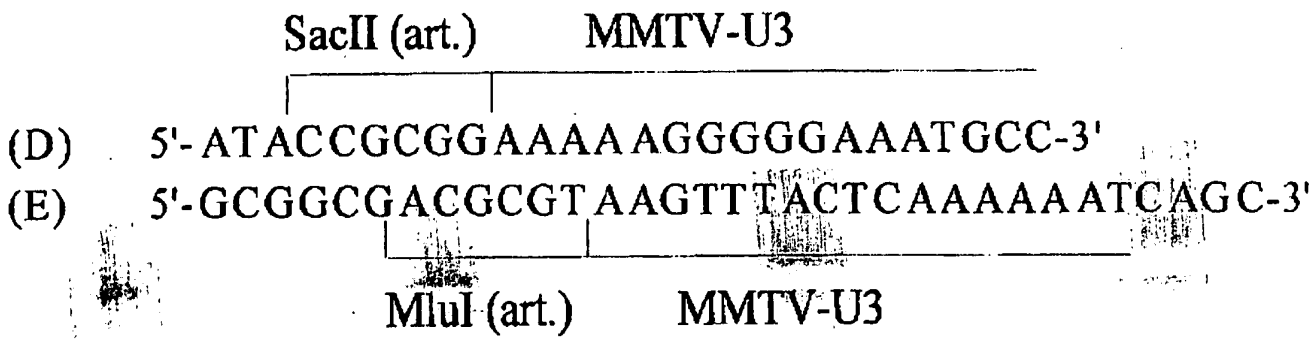
SacII (art.)
MMTV-U3
(D) 5'-ATACCGCGGAAAAAGGGGGAAATGCC-3'
(E) 5'-GCGGCGACGCGTAAGTTTACTCAAAAAATCAGC-3'
MluI (art.)
MMTV-U3

Fig. 7

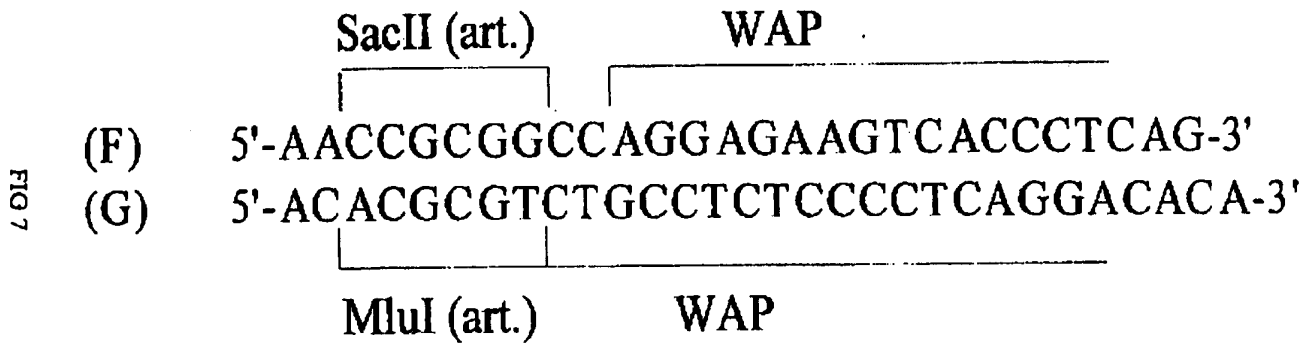
SacII (art.)
WAP
(F) 5'-AACCGCGGCCAGGAGAAGTCACCCTCAG-3'
(G) 5'-ACACGCGTCTGCCTCTCCCCTCAGGACACA-3'
MluI (art.)
WAP

FIG 7

Fig. 9

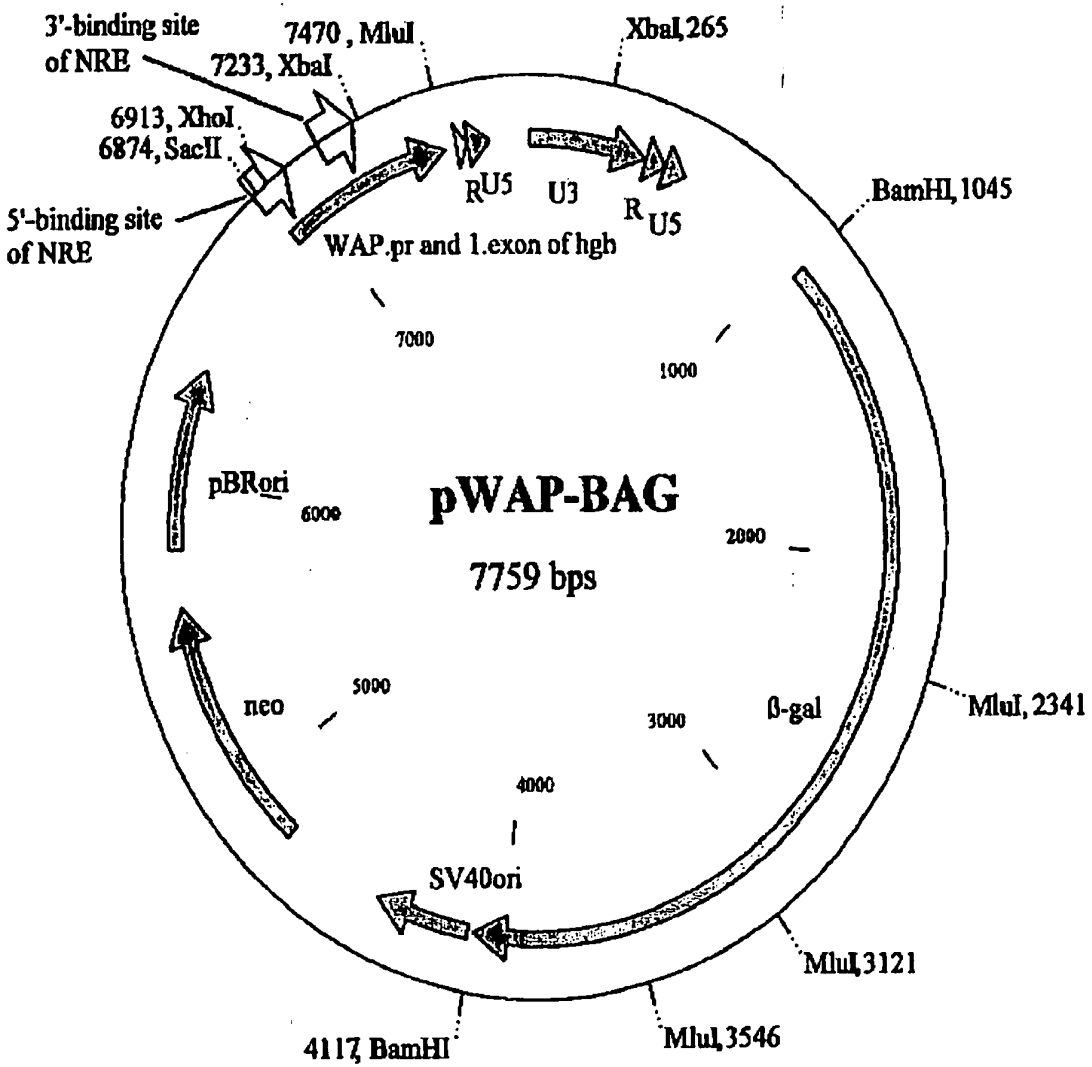
3'-binding site
of NRE
7470 , MluI
XbaI,265
7233, XbaI
6913, XhoI
6874, SacII
5'-binding site
of NRE
RU5
U3
R U5
BamHI,1045
WAP.pr and 1.exon of hgh
7000
1000
pBRori
pWAP-BAG
6000
2000
7759 bps
neo
5000
3000
β-gal
MluI,2341
4000
SV40ori
MluI,3121
4117, BamHI
MluI,3546

Fig. 10

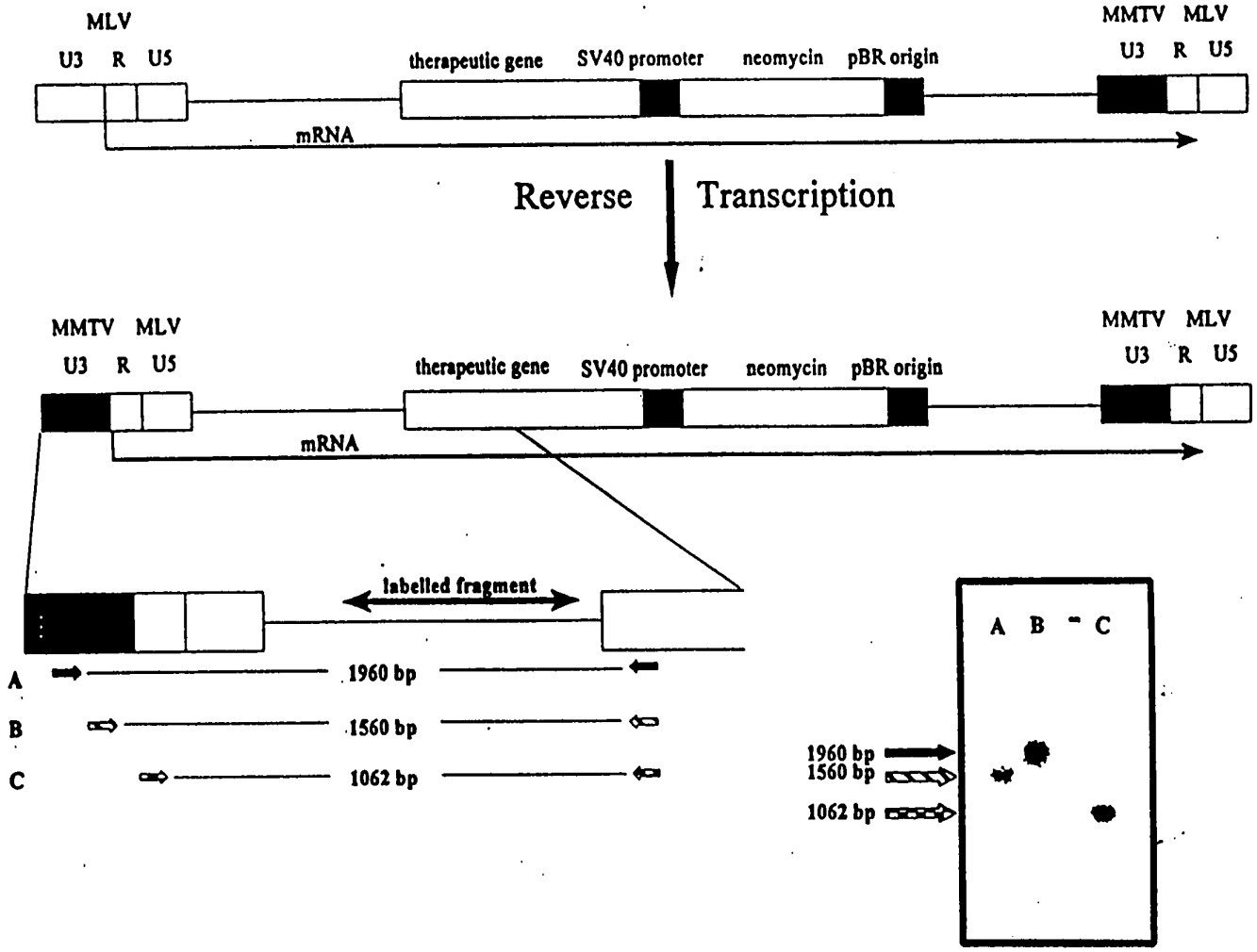
MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
MMTV MLV
U3 R U5
mRNA
Reverse Transcription
MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
MMTV MLV
U3 R U5
mRNA
labelled fragment
A
1960 bp
B
1560 bp
C
1062 bp
A B C
1960 bp
1560 bp
1062 bp

Fig. 11

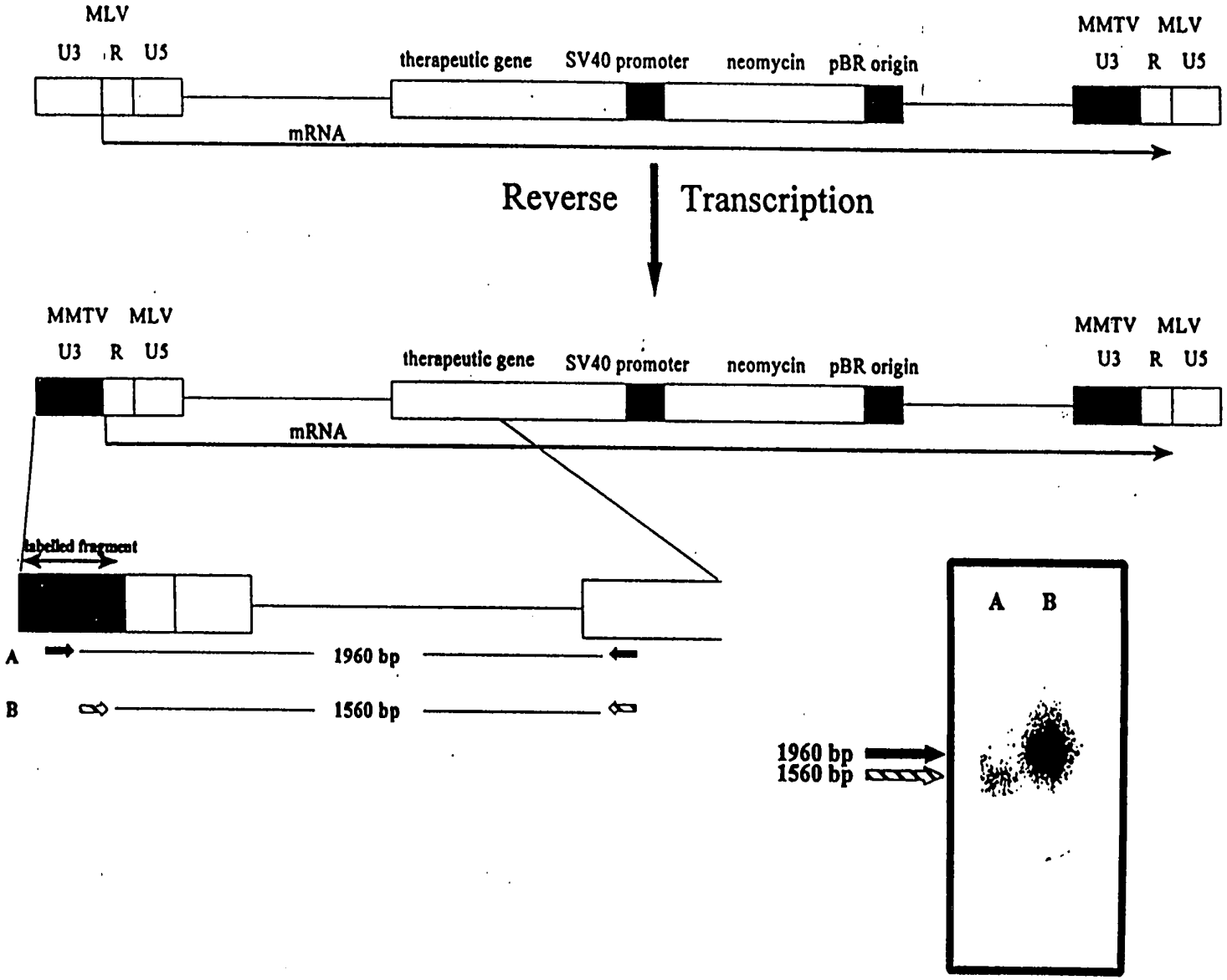
MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
MMTV MLV
U3 R U5
mRNA
Reverse Transcription
MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
MMTV MLV
U3 R U5
mRNA
labelled fragment
A
1960 bp
B
1560 bp
A B
1960 bp
1560 bp

Fig. 12

Z68220" Z2880880

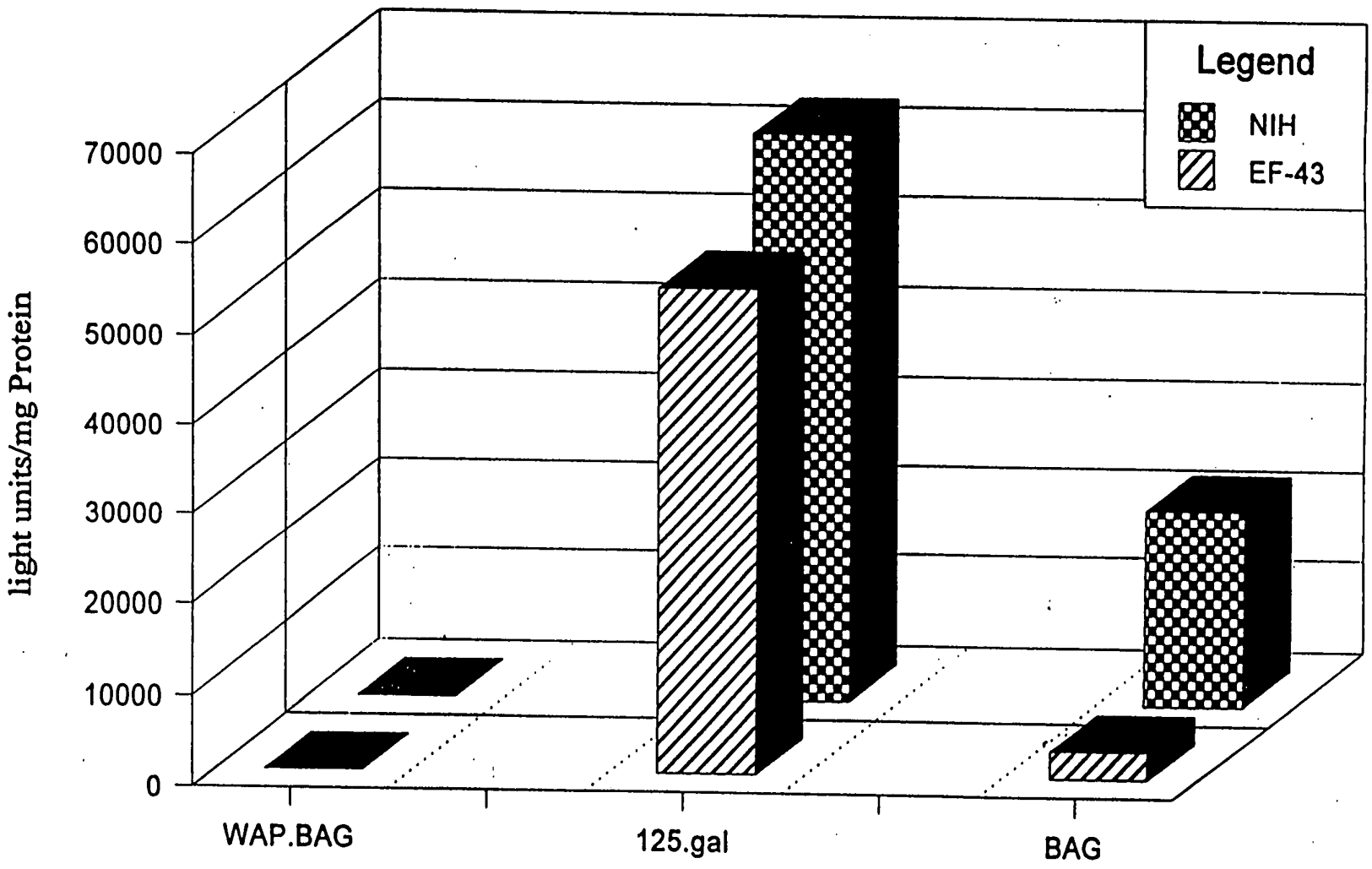

Fig. 13

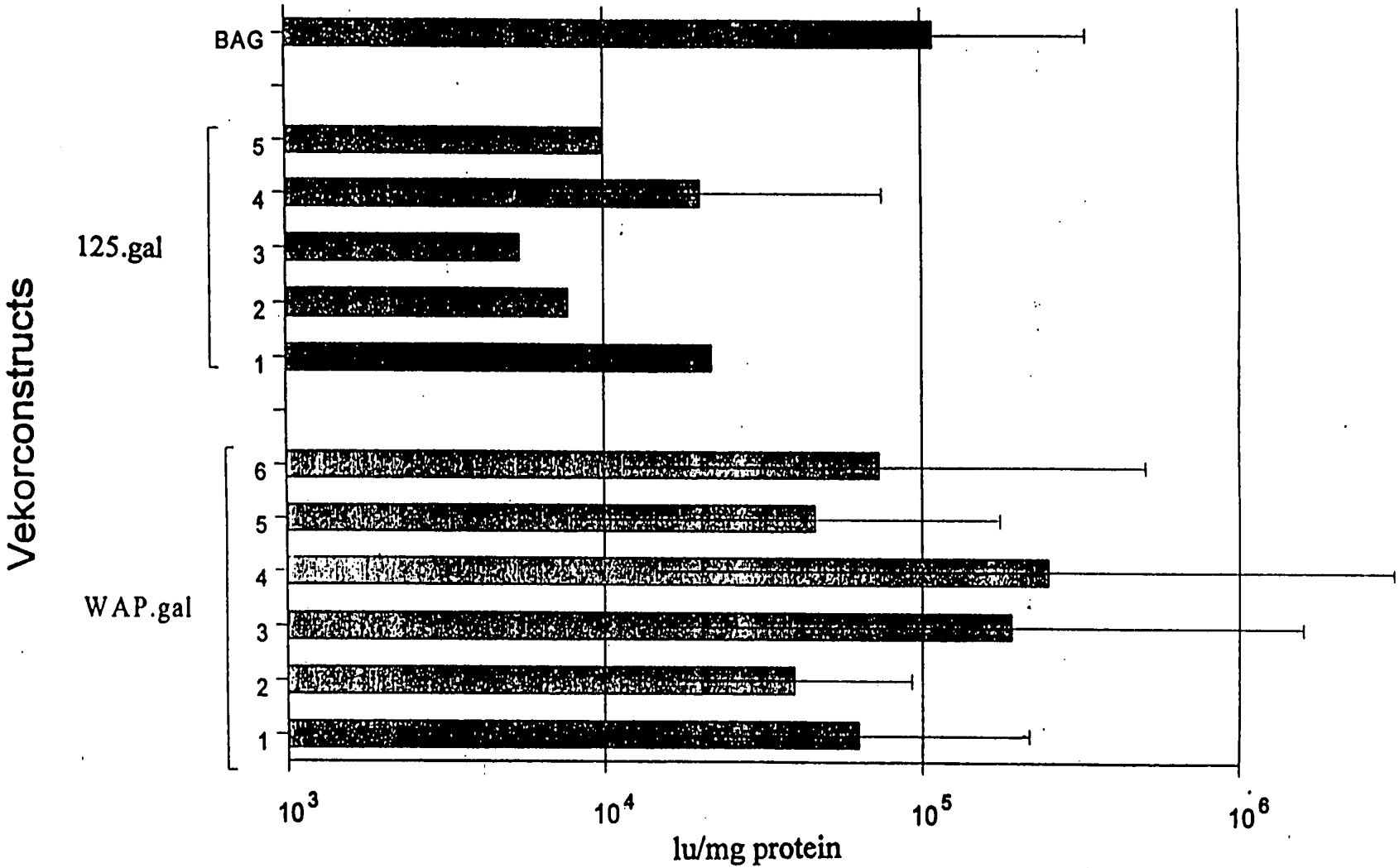
Vekorconstructs
BAG
125.gal
5
4
3
2
1
WAP.gal
6
5
4
3
2
1
10^3
10^4
10^5
10^6
lu/mg protein

Fig. 14

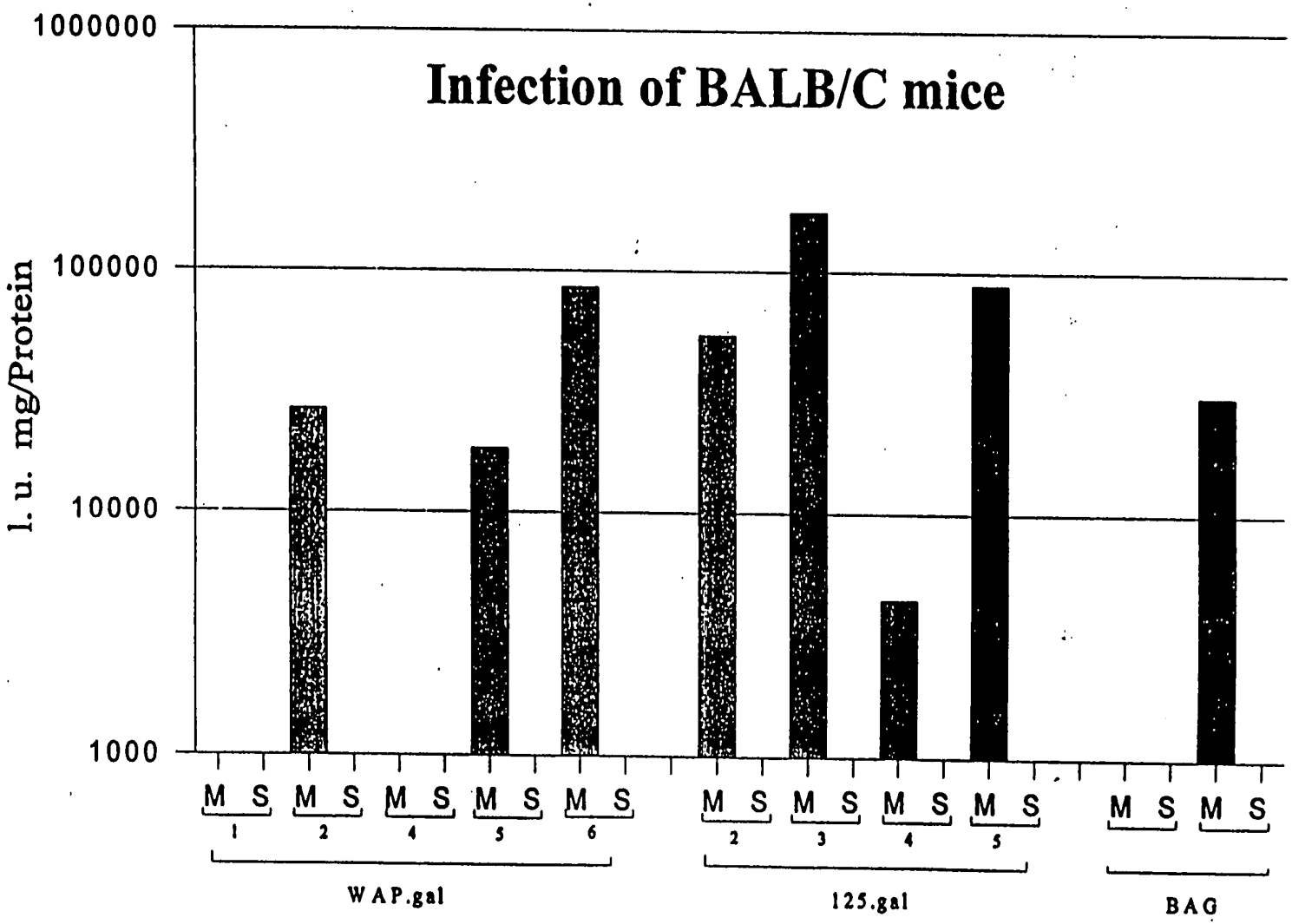
Infection of BALB/C mice
1000000
100000
10000
1000
l. u. mg/Protein
M S M S M S M S M S
1 2 4 5 6
WAP.gal
M S M S M S M S
2 3 4 5
125.gal
M S M S
BAG

Fig. 15

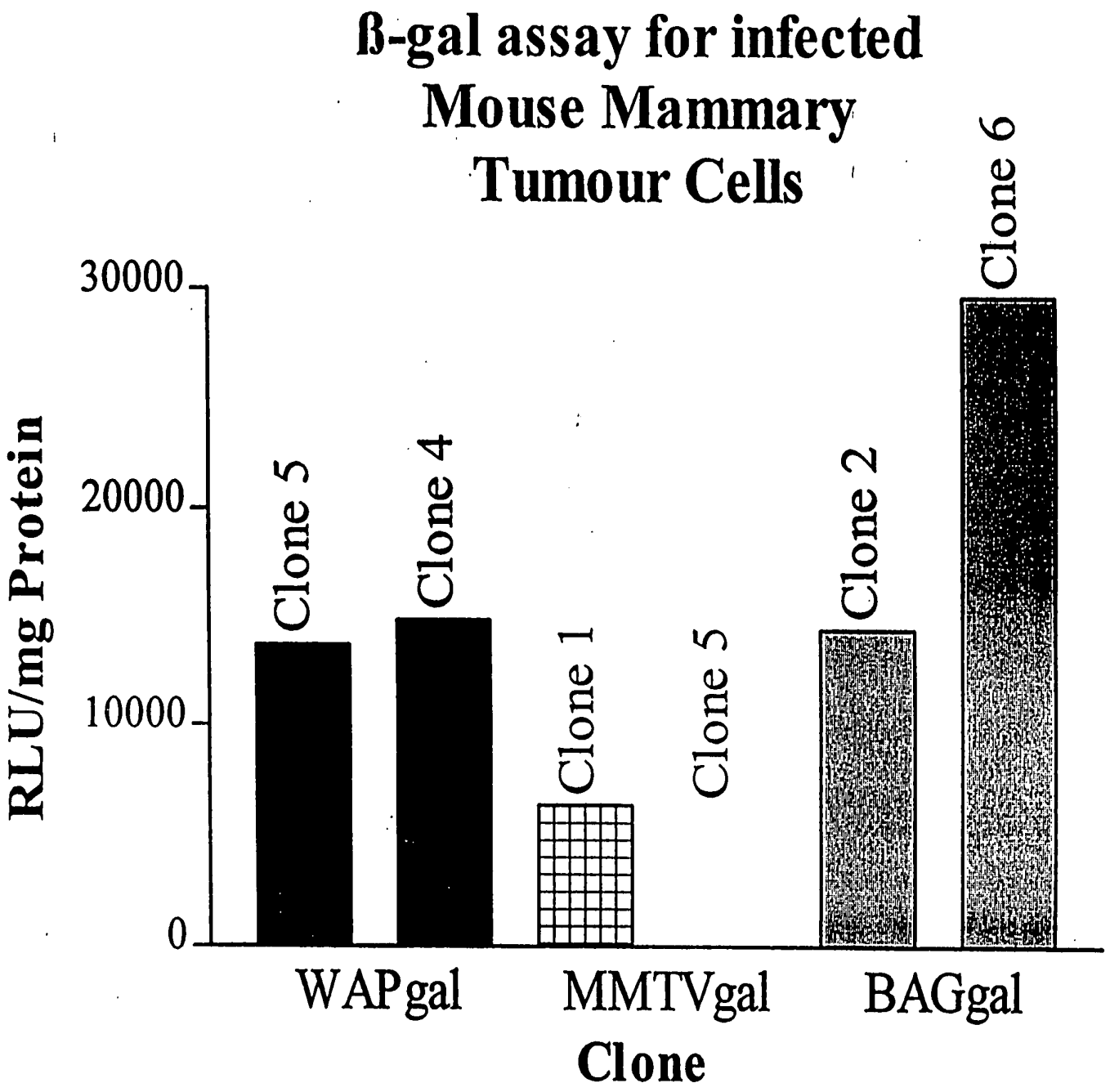
ß-gal assay for infected Mouse Mammary Tumour Cells
RLU/mg Protein
30000
20000
10000
0
Clone 5
Clone 4
Clone 1
Clone 5
Clone 2
Clone 6
WAPgal
MMTVgal
BAGgal
Clone

Fig. 16

Z68220 Z2880880

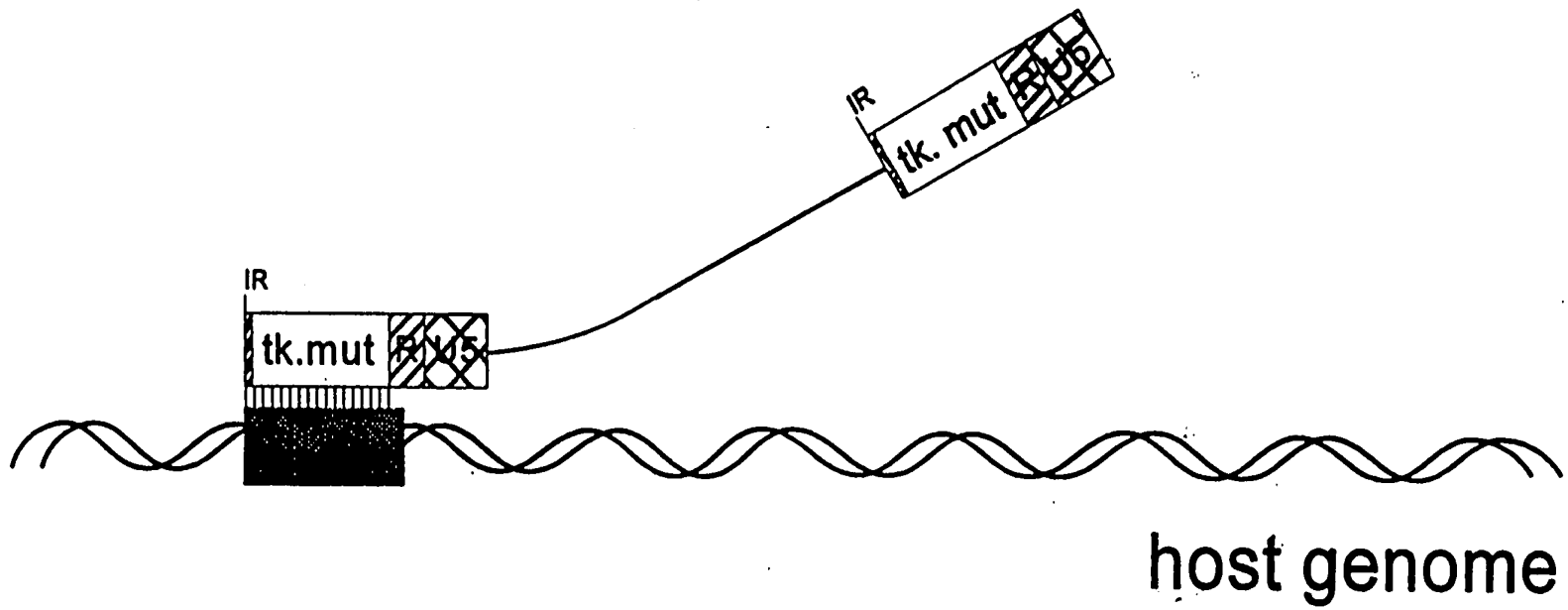

Fig. 17